



Gravitactic orientation of *Euglena gracilis*—a sensitive endpoint for ecotoxicological assessment of water pollutants

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Pollution of aquatic environments with natural and anthropogenically produced substances is one of the major environmental problems of the world. In many countries the decreasing quantity of water coupled with its increasing usage in multiple sectors has adversely affected water quality and caused problems of water pollution. Polluted water has been a main cause of adverse effects on plants, animals and humans throughout the world. Physicochemical analysis of water, which is a common method used for quality assessment of water, alone may not be enough as it cannot evaluate the impact on living organisms. Therefore, bioassessment of water and wastewater quality is considered to be essential to reflect the ultimate effects on living organisms. Many organisms like bacteria, algae, fish, invertebrates and protozoan are used as bioassay organisms for assessment of water quality. This review article elucidates the use of *Euglena gracilis*, a freshwater motile flagellate of the phylum Euglenophyta, as a suitable organism in ecotoxicological studies with special emphasis on its gravitactic orientation as a sensitive end point in ecotoxicological assessment of water pollutants.

Keywords: bioassessment, ECOTOX, ecotoxicology, *Euglena gracilis*, gravitactic orientation, water pollution

INTRODUCTION

About 70% of the earth surface is covered by water, of which less than 3% is fresh water and the remaining is saline. Due to factors like rapid increase in population, urbanization, industrialization and huge consumption of water in industry and agriculture, an immense stress on the quantity and quality of the available freshwater has been developed. In many countries the increasing usage of water in multiple sectors coupled with decreasing availability has adversely affected the quality of water and resulted in problems of severe water pollution. Polluted water has been among the main causes of various health problems in humans throughout the world. An estimate reveals that about 2.3 billion people in the world are suffering from diseases that are linked to water contamination (UNESCO, 2003). Every year, more than 2.2 million people across the developing world die from diseases related to inadequate sanitation and unclean water (WHO and UNICEF, 2000). In addition to health problems in humans, contaminated water has been reported as a cause of the death of aquatic organisms and livestock as well as has adverse effects on biodiversity and agricultural production (Nasrullah et al., 2006).

For the evaluation of water and wastewater quality, analytical methods are usually applied to evaluate physicochemical characteristics of water samples; however, these are expensive, may not cover all the toxic substances present in a sample and do not reflect the adverse effects on biota (Streb et al., 2002). In many countries, particularly in the developed world, wastewater treatment and cleaning is obligatory before it is discharged.

But even after treatment, many toxic substances may still be present in wastewater in small quantities, which can have possible adverse effects on living organisms. However, these effects on living organisms cannot be assessed by chemical analysis techniques (Danilov and Ekelund, 2000). Therefore, assessment of water and wastewater quality using bioassays is important to reflect the ultimate effects on living organisms. The main objective of this review is to describe *Euglena gracilis* as an ideal organism in ecotoxicological studies with special emphasis on its gravitactic orientation as a sensitive end point parameter.

BIOASSESSMENT OF WATER QUALITY

Many bioassays based on different organisms like bacteria, algae, fish, invertebrates and protozoa are being used for the assessment of water quality. One of the commonly used bioassays is that based on the changes in the bioluminescence of the bacterium *Vibrio fischeri* upon exposure to toxic substances. Due to the short response time, the bioluminescence tests have been widely used for toxicity assessment of various toxic substances in fresh and marine waters (Boyd et al., 2001; Vetrova et al., 2002; Wang et al., 2002). Standard test devices based on the luminescence of this bacterium like MICROTOX (Strategic Diagnostics Inc., Newark, USA) and LUMISTOX (Dr. Lange, Berlin, Germany) have been developed for a fast analysis of water samples (Kaiser, 1998; Streb et al., 2002).

Crustaceans such as *Daphnia* and *Ceriodaphnia* are also being widely used as biotest organisms. Toxicity assessment with these

organisms is mostly based on the immobilization of the organisms upon exposure to the test substance but the survival is also used as an end point. For example, Kline et al. (1989) performed the immobility tests with three species of cladocerans, namely *Daphnia pulex*, *Daphnia magna*, and *Ceriodaphnia dubia* to evaluate the toxicity of the pesticide triphenyltin hydroxide. The immobility tests with *Daphnia magna* and *Ceriodaphnia cf. dubia* were also applied for acute toxicity assessment of various detergents and detergent components (Warne and Schifko, 1999; Pettersson et al., 2000). Similarly, Chen and Liu (2008) evaluated the toxicity of the fungicide metalaxyl using 48 h mortality tests with *Daphnia magna*.

Fish are also known to be very vulnerable to pollutants and are used as sensitive test organisms in toxicity assessment of chemicals in aquatic environments. Behavioral responses of fish like swimming performance have commonly been used as end points in evaluating the toxic effect of toxicants. A video analysis system has been designed which investigates the stress or toxicity by recording changes in the behavioral parameters of fish like velocity, percent movement, total distance traveled, space utilization, angular change and fractal dimension (Kane et al., 2004). Bioassays based on the electric organ discharges (EOD) of fish species like *Apteronotus albifrons*, *Gnathonemus petersi* and *Gnathonemas tamandua* have also been developed (Geller, 1984; Lewis et al., 1995; Thomas et al., 1996).

Higher plants are also applied in the assessment of toxic substances. A common bioassay used for assessing pollutants in aquatic environment involving higher plants is that with duckweed, a group of flowering floating plants that belong to the family *lemnaceae* (Wang, 1990). Due to characteristics like small size, widespread occurrence, fast growth and high sensitivity to toxic substances, duckweed is accepted as an ideal organism for bioassessment in aquatic environments (Wang, 1990). Various active pollutants of aquatic environments like heavy metals, phenolic compounds and pesticides have been assessed for their toxicity potential using duckweed species (Wang, 1990; Mohan and Hosetti, 1997; Hou et al., 2007; Paczkowska et al., 2007).

Among the various bioassay organisms used in aquatic ecotoxicology, algae are considered to be more suitable organisms as they have higher sensitivity to aquatic pollutants and are the abundantly occurring organisms in aquatic environments (Danilov and Ekelund, 2000; Ahmed and Häder, 2010a). An advantage of algal biotests is that they are easy to perform and allow observing multiple generations. Algal bioassays also help avoiding the use of experimental animals which is desirable for economical and ethical reasons (Millán de Kuhn et al., 2006). Diverse species of algae belonging to different groups, e.g., *Chlorella*, *Laminaria*, *Raphidocelis*, *Scenedesmus*, and *Selenastrum* have been used for bioassessment of fresh and marine water pollutants (Pybus, 1973; Christensen et al., 1979; Anton et al., 1993; Ma et al., 2002; Pavlic et al., 2005). Different algal parameters like cell number, fresh or dry weight, protein and nucleic acid contents, chlorophyll fluorescence, CO₂ fixation, ATP production, morphology or vital stainability are being used as endpoints in ecotoxicology (Rai et al., 1994).

***Euglena gracilis*—AS A BIOASSAY ORGANISM**

E. gracilis is a unicellular motile flagellate of the phylum Euglenophyta and is found in many freshwater aquatic habitats, especially shallow eutrophic ponds (Tahedl and Häder, 2001). Due to its rapid and sensitive response to various toxic substances like heavy metals and inorganic and organic compounds, *E. gracilis* has been among the widely used organisms in ecotoxicological studies. Numerous behavioral, biochemical, morphological and physiological parameters of *Euglena* are being used as end points in biomonitoring of water pollutants. For example, cell growth in *Euglena* has been used in numerous studies to evaluate the effects of different substances (Gajdosova and Reichrtova, 1996; Aronsson and Ekelund, 2005). Its motility, orientation and morphological parameters like percent motility, swimming velocity, upward swimming and cell shape have been widely used for assessing the toxicity of water pollutants of both organic and inorganic nature including wastewaters (Tahedl and Häder, 1999; Pettersson and Ekelund, 2006; Azizullah et al., 2013). Photosynthetic efficiency and composition of photosynthetic pigments in this flagellate were found to be sensitive to different chemicals and have been used as end points in assessing the impact of toxic chemicals (Nass and Ben Shaul, 1973; Ahmed, 2010; Azizullah et al., 2012).

GRAVITAXIS IN *E. gracilis*—A SENSITIVE END POINT IN ECOTOXICITY ASSESSMENT

E. gracilis has a flagellum inserted at the front end which is used as a locomotory organ (a second but rudimentary and non-functional small flagellum is also present but it does not protrude out of the frontal invagination) (Häder, 1987; Tahedl and Häder, 1999). *E. gracilis* uses light and gravity as external clues for orienting itself in the water to reach a region in the water column optimal for reproduction and growth (Häder, 1987; Richter et al., 2003). Both gravitaxis and phototaxis in *Euglena* are based on active physiological mechanisms (Kamphuis, 1999; Richter et al., 2001), and have been found to be impaired by external stressors such as heavy metals, organic and inorganic pollutants, increased salinity, and ultraviolet radiation (Tahedl and Häder, 1999; Richter et al., 2003). Various investigations led to the conclusion that the difference in density between the cell body and the surrounding medium is an important factor in the gravitactic orientation of *Euglena* (Lebert et al., 1999). Since the density of the cell body is higher than the surrounding medium, it causes a downward sedimentation of the cell contents which stretches the lower membrane by exerting a pressure on it. This stretching results in the activation of mechano-sensitive ion channels in the cell membrane and ultimately changes the membrane potential which triggers a reorientation of the flagellar movement (Lebert et al., 1999). The application of low concentrations of mechano-sensitive ion channel inhibitors, such as gadolinium, strongly impaired the gravitactic orientation which revealed the involvement of mechano-sensitive ion channels in gravitactic orientation (Franco et al., 1991). The pollutants present in a water sample affect these mechano-sensitive ion channels and thus cause impairment of the gravitactic orientation in *Euglena* cells (Tahedl and Häder, 1999).

With the development of the automatic bioassay device ECOTOX (Tahedl and Häder, 1999, 2001), which uses motility and orientation parameters of *Euglena* as end points, gravitactic orientation in this flagellate has widely been accepted and applied in ecotoxicological assessment of aquatic pollutants of diverse nature. For example, it was successfully applied in the toxicity assessment of heavy metals (Ahmed, 2010), organic pollutants (Tahedl and Häder, 1999, 2001), fertilizers (Azizullah et al., 2012), pesticides (Pettersson and Ekelund, 2006; Azizullah et al., 2011a,b,c,d), detergents (Azizullah et al., 2011a,b,c,d) and wastewater (Ahmed, 2010; Azizullah et al., 2013). In short-term tests, the gravitactic orientation in *E. gracilis* has been reported to be more sensitive toward wastewaters and many other pollutants as compared to other motility parameters of the organism (Tahedl and Häder, 1999; Ahmed, 2010; Azizullah et al., 2011a,b,c,d, 2012). The presence of mechano-sensitive ion channels in the cell membrane of *Euglena* and their role as gravireceptors (Häder et al., 2009) has been regarded as a probable reason for the higher sensitivity of gravitaxis in *Euglena* to aquatic pollutants (Ahmed and Häder, 2010b). Previous studies also revealed that motility and orientation in *E. gracilis* were more sensitive than photosynthesis (measured by chlorophyll fluorescence) to different pollutants (Ahmed, 2010; Azizullah et al., 2011a,b,c,d, 2013). Based on the assessment of many wastewater samples collected from different industries by our group, we have concluded that precision of gravitactic orientation in *E. gracilis* is the most sensitive parameter toward wastewater toxicity during short-time tests (immediately after exposure) (Azizullah et al., 2011b, 2012, 2013). These parameters were also found to be more sensitive than other common biotests such as algal growth test, *Daphnia* motility test, fish mortality test, and bacterial bioluminescence test (MICROTOX) to wastewater toxicity from different industries (Ahmed and Häder, 2011).

Gravitactic orientation, in combination with other motility parameters of *Euglena*, makes ECOTOX an ideal system for ecotoxicological assessments in aquatic environments. In comparison to other commonly used biotests, low costs and short time for measurement are the main advantages of ECOTOX. Depending upon setting of the software, 6–10 min are needed for one complete measurement of a sample along with the corresponding control. Automated measurement and data analysis and the usage of multiple parameters as end points are other advantages of ECOTOX (Azizullah et al., 2011b).

CONCLUSIONS

Pollution of aquatic environments from natural and anthropogenic sources is one of the major environmental problems of the world. Physicochemical analysis of water alone may not be enough as it does not reflect the adverse effects on living organisms. The involvement of bioassays is necessary in pollution monitoring of aquatic environments. *E. gracilis* is an ideal organism for use in ecotoxicological studies with its various physiological, biochemical and behavioral parameters used as end points. The automatic biotest ECOTOX is a value tool in pollution monitoring. Among its various end point parameters, gravitactic orientation of *Euglena* is recommended as the most sensitive end point in

short-term (immediately upon exposure) assessment of aquatic pollutants.

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